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REACTIVATION OF IRRADIATED BACTERIOPHAGE BY TRANSFER OF SELF-REPRODUCING UNITS

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In investigating certain peculiarities in the titration of the activity of bacteriophages after ultraviolet irradiation, a mechanism was discovered that offers direct support for interpreting inactivation of these viruses by radiation as due to lethal mutations. Moreover, this mechanism reveals unexpected features of the process of virus reproduction. We summarize here the results of this work and discuss some of their implications. They will be published in detail elsewhere.

Our work employed the coli bacteriophages, T1–T7, their "r" mutants (i.e., with rapid lysis, and large plaque), the host strain, *Escherichia coli* B, and a number of bacterial mutants resistant to one or another of the bacteriophages.[1, 2]

The rate of inactivation of these bacteriophages exposed to ultraviolet light (wave-length 2537 A) in a non-absorbent medium is a simple logarithmic function of the dose of irradiation (Fig. 1).[3,4] This indicates a "one hit" mechanism of inactivation, one quantum being the effective inactivating hit. The phage survival is generally measured by plaque count, by diluting the irradiated phage rather heavily in broth and plating the diluted samples with sensitive bacteria. Each phage particle that remains active will give one plaque (phage colony).

It was, however, noticed by Delbrück and Bailey (personal communication) that the active titre of an irradiated suspension, as determined by plaque count, is dependent on the concentration of the irradiated sample when first placed in contact with the bacterial cells. For example, an irradiated sample can be diluted 1:10 into a heavy bacterial suspension, and after a few minutes—during which no phage liberation occurs—diluted again $1:10^4$ in broth and plated. The count will be much higher than if the same sample had first been diluted $1:10^4$ in broth, then 1:10 in bacterial suspension. This phenomenon seemed to indicate the presence in irradi-

ated phage suspensions of some "factor," besides the phage itself, capable of causing reactivation of inactivated phage particles if allowed to act on the same bacterial cells. A study of this factor was the original purpose of the work reported here.

It has been shown previously,[5] and again in the experiments discussed in this paper, that ultra-violet inactivated phage particles are still adsorbed by sensitive bacteria at the same rate as active particles, even after having received as many as 40–50 lethal hits. This can be demonstrated because

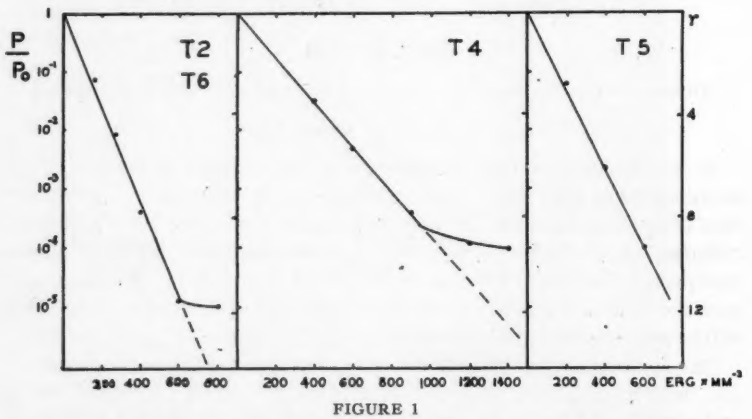

FIGURE 1

Survival curves for phages T2, T6, T4, T5 in synthetic medium. P/P_0 = proportion of active phage particles after irradiation. $r = \ln P_0/P$ = average number of lethal hits per particle. The deviations for high doses are due to the reactivation (described in this paper) taking place on the assay plates.

each bacterial cell that adsorbs one phage particle fails to divide and to produce a colony after plating. Incidentally, this fact makes it possible to calculate the number and rate of adsorption of ultraviolet inactivated phage particles. The unknown reactivating "factor" must be something that, when acting on a bacterial cell that has adsorbed an inactive phage particle, causes the production of active phage.

We first established that reactivation only occurs for the large-particle phages T2, T4, T6, T5, and their mutants, not the small phages T1 and T7. It was found next that the "factor" is partially phage specific, in the sense that even a concentrated suspension of one phage can generally not reactivate the particles of another irradiated phage. This can be tested by preparing mixtures of host cells with two phages in various proportions, the irradiated phage whose reactivation is to be tested being so diluted that

little or no reactivation would take place in the absence of the other phage suspension. After a few minutes of contact, the mixtures are diluted and plated with bacteria sensitive only to the phage whose reactivation is being tested. Since all phage suspensions are lysates of the same host cells, the specificity of reactivation suggests that the "factor" is not of bacterial origin.

An important exception to specificity is that cross-reactivation occurs between suspensions of phages of the T-even group (T2, T4, T6). These phages, although representing different wild types and distinguishable by a number of characteristics, are known to be serologically related and morphologically similar. Moreover, particles of these phages are known to be capable of mutual transfer of hereditary characteristics when adsorbed by the same host cell.[6] Reactivation of one of these T-even phages can be induced both by irradiated and non-irradiated suspensions of any of the other T-even phages.

These results suggested that the reactivating "factor" might simply be phage itself, in the sense that an inactive phage particle, if adsorbed on the same cell with another particle of the same or of a related strain, could be reactivated by transfer from the latter of the genetic locus or loci at which lethal mutations had occurred.

This hypothesis was qualitatively supported by the finding that cross-reactivation between two related phages can only occur in the presence of bacteria capable of adsorbing both phages, not in the presence of bacteria sensitive only to one of them. For example, T6 can reactivate T2 in presence of strain B, sensitive to both, but not in presence of bacteria B/6, sensitive to T2 and resistant to T6.

That reactivation is not caused by some other "factor" in the lysates was also supported by the finding that reactivation occurred with phage that had been purified from extraneous material by differential centrifugation (Strain T4r, kindly supplied by Dr. T. F. Anderson).

For quantitative testing, our hypothesis can be formulated more exactly by stating that reactivation should only occur inside bacterial cells that adsorb two or more bacteriophage particles (multiple-infected bacteria). This expectation can be tested for each phage by using several mixtures of bacteria and irradiated phage in different proportions, and calculating for each mixture the number of bacteria that adsorb more than one phage particle. If the average number of phage particles adsorbed per cell is x, the proportion of cells with more than one phage particle is given by the expression: $1 - (x + 1)e^{-x}$. For x small, this expression corresponds very closely to the proportion of cells with two phage particles. The values for multiple-infected bacteria thus obtained can then be compared with the actual numbers of bacteria that liberate phage in each mixture. These numbers are measured by plating a sample for phage plaque count before lysis

TABLE 1

RELATION OF THE NUMBER OF BACTERIA YIELDING PHAGE TO THE NUMBER OF MULTIPLE-INFECTED BACTERIA

Series of mixtures containing the same number of bacteria and different numbers of phage particles were kept 10 minutes at 37°C., then plated for plaque count. The number of multiple-infected bacteria in each mixture was calculated from the formula

$$[B > 1P] = [1 - (x + 1)e^{-x}][B],$$

where $[B]$ = total number of bacteria per ml.; $[B > 1P]$ = multiple-infected bacteria per ml.; x = ratio "adsorbed phage/bacteria" in each mixture.

Expt. No.	41			43			38		
Phage	T4			T4			T6		
Dose, erg × mm.$^{-2}$	600			900			500		
x	$[B > 1P]$ (Calc.)	Count (Exper.)	Ratio $1/y$ (Calc.)/(Exper.)	x	$[B > 1P]$ (Calc.)	Count (Exper.)	Ratio $1/y$ (Calc.)/(Exper.)	x	$[B > 1P]$ (Calc.)	Count (Exper.)	Ratio $1/y$ (Calc.)/(Exper.)
0.67	1.7×10^6	9×10^7	1.9	0.95	1.9×10^8	1.7×10^7	11	0.62	1.6×10^8	3.9×10^7	4.1
0.33	5.0×10^7	3×10^7	1.8	0.48	6.5×10^7	5.4×10^6	12	0.31	3.6×10^7	1.2×10^7	3.0
0.135	1.1×10^7	6×10^6	1.9	0.24	1.9×10^7	1.5×10^6	13	0.155	1.3×10^7	3.2×10^6	4.1
0.067	2.6×10^6	1.3×10^6	2.0	0.09	3.2×10^6	2.2×10^5	14	0.062	2.4×10^6	6.7×10^5	3.6
0.033	6.8×10^5	3×10^5	2.1	0.05	9.0×10^5	8.0×10^4	11	0.031	5.4×10^5	1.3×10^5	4.2
0.013	1.7×10^5	1×10^5	1.7					0.015	1.7×10^5	4.1×10^4	4.1
	Av.		1.92		Av.		12.2		Av.		3.85
	σ		0.16		σ		1.2		σ		0.46

occurs; every bacterium that liberates active phage will produce one plaque.

If the hypothesis is correct, the plaque counts should be directly proportional to the numbers of multiple-infected bacteria, the factor of proportionality depending on the dose of irradiation in a way that will be discussed later. A large number of experiments of this type gave results agreeing remarkably well with the expectation. Representative results are given in table 1. It was also found in these experiments that bacteria infected with more than one inactive phage particle liberate a roughly normal yield of fully active particles.

Our hypothesis can now be elaborated to give expectations for the actual values of the factor of proportionality between the numbers

of multiple-infected bacteria and of bacteria that yield active phage. We shall assume that a phage particle contains a certain number of different self-reproducing "units" (loci), each capable of undergoing a lethal mutation under the action of radiation. Reactivation depends on transfer of these units, the requirement for reactivation being that a given locus does *not* carry a lethal mutation in *all* of the particles that infect the same cell. The probability that this requirement is satisfied will depend on the dose of radiation, since the probability that a lethal hit takes place at the same locus in all infecting particles will increase with increasing doses of radiation. This hypothesis is graphically described in figure 2. It can be formulated mathematically by assuming that there are in each particle of a given phage n units each capable of giving a lethal mutation. We further assume, in first approximation, that the sensitivity of all units to radiation is the same.

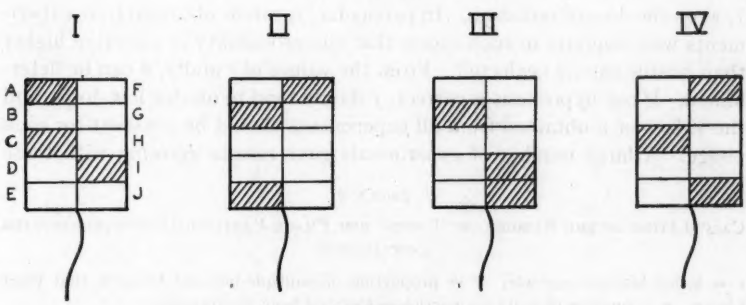

FIGURE 2

Schematic representation of phage particles, each carrying three lethal mutations, distributed at random among ten loci.

A bacterium infected with particles (I + II) will yield active phage.
A bacterium infected with particles (I + III) will not yield active phage.
A bacterium infected with particles (I + IV) will not yield active phage.
A bacterium infected with particles (III + IV) will not yield active phage.
A bacterium infected with particles (I + III + IV) will yield active phage.

The probability y_k that none of the n units carries a lethal mutation in all of k particles adsorbed by the same cell is then found[7] to be

$$y_k = [1 - (1 - e^{-r/n})^k]^n \qquad (1)$$

where r is the average number of lethal hits or mutations per particle. The value of r can be read directly from the regular survival curve, as given in figure 1, r being the natural logarithm of the ratio between the initial titre and the survival: $r = \ln P_0/P$.

For $r/n \ll 1$—that is, for low doses of radiation—the formula (1) can be simplified with good approximation to

$$y_k \approx e^{-r^k/n^{k-1}} \qquad (1')$$

In particular, for bacteria that adsorb only two phage particles ($k = 2$), the probability becomes

$$y_2 = [1 - (1 - e^{-r/n})^2]^n \qquad (2)$$

$$y_2 \approx e^{-r^2/n} \qquad (2')$$

It must be noticed that for low doses, when the probability that a lethal mutation at any given locus occurs in all particles is small, the values of y should tend to one if the efficiency of the transfer mechanism is complete, that is, if there is full recombination of active units to form active phage particles.

The formulas (1, 1′) and (2, 2′) can be tested experimentally by studying the dependence of the probability of reactivation (see table 1, columns $1/y$) on the dose of radiation. In particular, y_2 can be obtained from experiments with bacteria in such excess that the probability of infection higher than double can be neglected. From the values of r and y, n can be determined. If our hypothesis is correct, y should tend to one for low doses, and the values of n obtained from all experiments should be constant for each phage. A large number of experiments gave results agreeing with these

TABLE 2

CALCULATION OF THE NUMBER OF "UNITS" PER PHAGE PARTICLE (EXPERIMENTS WITH LOW DOSES)

r = lethal hits per particle; y = proportion of multiple-infected bacteria that yield phage; n = number of units per particle calculated from the formula

$$y = [1 - (1 - e^{-r/n})^2]^n \approx e^{-r^2/n} \text{ (for } r/n \ll 1). \quad n = r^2/\ln(1/y).$$

PHAGE	r	$1/y$	n	PHAGE	r	$1/y$	n
T2–T2r	2.6	1	..	T6	3.6	1	..
	4.8	1.6	49		7.9	3.4	51
	7.7	3.5	47		9.4	5.7	50
	11.0	19	41		11.7	17	49
	14.3	93	45		12.6	40	43
		Av.	45.5		15.5	70	57
						Av.	50
T4–T4r	2.8	1	..	T5	2.8	1.9	12
	5.1	2.8	26		6.1	16	13
	7.8	5.7	36		9.2	67	19
	10.0	21	33			Av.	15*
		Av.	32				

* Values uncertain, due to very low adsorption rate of this phage.

expectations. Some experiments are shown in table 2, where $1/y$ is given instead of y for convenience. We may then conclude that active phage is

formed from inactive particles by a highly efficient mechanism of transfer of any one of a relatively large number of independently transferable units: 45–50 for phages T2 and T6, 30–35 for phage T4, possibly around 15 for phage T5.

Formula (1) requires that the probability of reactivation increases with increasing values of k, that is, of the number of phage particles adsorbed per bacterium. This was verified by experiments in which bacteria were mixed with larger amounts of irradiated phage (x higher),[8] so that the probability of three or more phage particles being adsorbed by the same cell became appreciable. Representative data are given in table 3, and are seen to agree with the expectation that the values of y increase with increasing values of x (and of k).

TABLE 3

DEPENDENCE OF THE PROBABILITY OF REACTIVATION ON THE MULTIPLICITY OF INFECTION

DOSE, ERG × MM.$^{-2}$			400	600	800	1000
EXPT. NO.	PHAGE	x	$1/y$	$1/y$	$1/y$	$1/y$
71	T6	0.11	..	8.1	41	...
		0.55	..	7.8	25	130
		1.1	..	4.1	18	44
		2.7	..	5.1	15	44
77	T2	0.39	2.6	10.6	60	...
		0.78	2.4	9.3	35	...
		3.9	2.0	3.4	10	...
		7.8	1.65	2.15	5.3	...

These data permitted us to obtain a rough independent estimate of n from the probability of reactivation in bacteria with more than two phages. The values thus obtained for n are in sufficient agreement with those given in table 2, although generally a little lower. This systematic deviation seems to indicate that the efficiency of transfer in the case of infection with more than two particles is lower than 100%.

Our next question is the following: Does all inactivation of phage particles by ultraviolet light occur through production of lethal mutations in transferable units, or will some other mechanism of inactivation appear for very high doses? This point was investigated by using heavy doses (up to 40–50 lethal hits per particle) and comparing the results with the expectations from formula (2) for double infection. Any additional mechanism of inactivation appearing at high doses should result in a systematic deviation in the sense of less reactivation than predicted by the formula. No such deviation was found; this indicated that, even for large doses of radiation, all effect can be accounted for by lethal mutations in the transferable units.

This conclusion also has another interesting feature: it indicates that there is no appreciable amount of "linkage" in the transfer process. Any tendency of units to be transferred in groups rather than individually should give a deviation in the direction of too low reactivation at high doses, when the probability of producing two or more lethals within a linkage group becomes appreciable.

The next step was an attempt to elucidate the mechanism of transfer. The obvious analogy with crossing-over might suggest that the two entering particles exchange units directly, before any multiplication takes place, and that what then multiplies is a fully active particle resulting by exchange. This possibility is difficult to reconcile with the very high efficiency of reactivation: in case of crossing-over we would hardly expect that all active units be collected into one active particle. Another test can be made by the cross described in figure 3. Remembering that an active particle of one phage may reactivate an inactive particle of another phage, we may expect that transfer will also occur between active and inactive particles of the same phage. If there is direct crossing-over, an active particle, if adsorbed with inactive ones onto the same cell, should often be inactivated by transfer of some "lethal" unit.

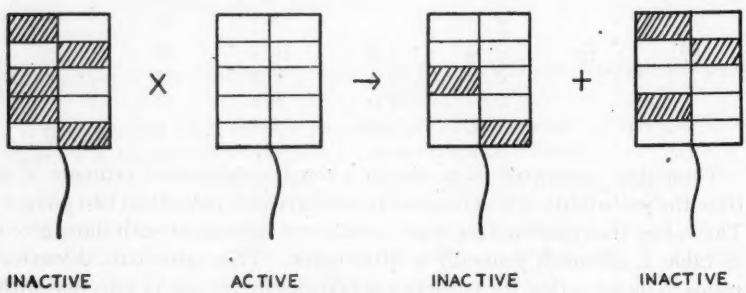

FIGURE 3

Schematic representation of the expectation in case of "cross" between a heavily irradiated particle and an active particle, assuming transfer of units by direct crossing-over between the two infecting particles.

In the actual experiments, phage suspensions were given large doses of radiation (20–50 hits per particle). Mixtures were set up containing bacteria, inactive phage, and active phage in proportion such that a large number of cells received one active and one or more inactive particles. If there were direct exchanges before reproduction, the result should be the one described in figure 3. A high proportion of the infected cells should end up with two inactive particles and, therefore, should yield no active

phage. This was not found to occur in any case: an active phage particle was never inactivated by "crossing" with an inactive one.[9]

A possible alternative interpretation of the transfer is that each of the active units can reproduce copies of itself in excess of the number needed for multiplication of the phage particle as a whole. The copies of each unit might then either become incorporated into other phage particles that missed them, or come together to reconstitute active phage particles, independently of the origin of the individual units from one or another of the infecting particles. We have no evidence as yet available whether the "lethal" units do not multiply, or multiply in a way that makes them unfit for their niche in the phage particle. In view of the high efficiency of the reactivation process and of the approximately normal yield of phage particles, it seems certain that, if the inactive units reproduce at all, they do not have any appreciable chance of becoming incorporated into the newly formed particles.

We also have no evidence as yet as to whether the active units in an inactive particle will proceed to multiply in single-infected cells. The same may be said for the question whether the active units remain in spatial relation to each other, as it were, in a "frame phage particle," while reproducing excess copies that are incorporated into the new particles that are formed. Work to attack some of these problems is now in progress.

A point of considerable interest is that in numerous experiments in which phage particles were inactivated by x-rays instead of ultraviolet light, no reactivation occurred, although the x-ray inactivated particles were still adsorbed by the bacterial cells.

Discussion: These experiments have suggested a possible mechanism of reproduction of phage particles inside the host cell, according to which reproduction would take place, as it were, in an "atomistic" way, by independent reproduction of a number of units and incorporation of these into the final phage particles. A number of problems may be raised, as to the nature of the self-reproducing units, the structure of the phage particle, and the relation of its mechanism of reproduction to that of other self-reproducing entities (genes, plasmagenes).

In the first place, each unit seems to react as a distinct photochemical entity in respect to ultraviolet quanta. The assumption of equal sensitivity is very likely unjustified; a variation in sensitivity from locus to locus would tend to make the calculated number of loci per particle too small, since the most sensitive units have a greater chance of being lethally hit earlier in all infecting particles. This would result in less reactivation and apparently lower number of loci. Our estimates of the number of loci are therefore minima. It is interesting to notice that phage T4, which is about twice as resistant to ultraviolet than the related phages T2 and T6, also appears to have fewer loci. Its higher resistance seems, therefore, to be due

to actual absence of a number of loci rather than to absence of some particularly sensitive locus.

Our results with the small phages T1 and T7, where no reactivation was detected, do not prove that these phages do not possess a number of independently transferable loci. They only indicate that, if such loci occur, they are too few to be detected in our experiments, that is, fewer than 8 or 10. These phages are actually about 2.5 times more resistant to ultraviolet than phage T4.

As for the failure of particles inactivated by ionizing radiation (x-rays) to undergo reactivation, this may be interpreted as indicating, either spread of the lethal effect of each ionization (or group of ionizations) to a large number of loci, or, less likely, an ability of the x-rays to cause other changes than those produced by ultraviolet. A projected study with various monochromatic radiations may throw some light on this question. It is interesting to notice that Lea and Salaman,[10] extending earlier work on the rate of inactivation of phage by ionizing radiation,[11] and analyzing their results in terms of the density of ionization, recently concluded that the "sensitive zone" of a large phage particle may be resolved into about fourteen units (genes?). Because of the assumptions involved in their calculations, their results by themselves can hardly be considered as more than qualitative indication of a geometrically complicated organization of the radiation sensitive material of the phage particle. In the light of our experiments, however, the interpretation offered by Lea and Salaman appears quite plausible, at least in assuming a multiplicity of sensitive units.

The incorporation of discrete units into organized phage particles is not easy to visualize, in view of the complex structure and morphology of the latter.[12] We can imagine that each active unit impresses its specificity on a number of elements produced in excess inside the host cell, elements which represent the raw material then utilized to build more phage particles. The units carrying lethal mutations may be unable to mold the substrate in their own image and likeness.

It is plausible, although not yet experimentally demonstrated, that some of the transferrable units that can undergo lethal mutations are the same gene-like entities responsible for determination of the transferable characters studied by Delbrück and Bailey[6] and by Hershey[18].

The mechanism of transfer suggested for bacteriophages appears to differ from that of crossing-over by being a transfer of units by "infection" rather than an "exchange" of portions of gene strings. One might, however, conceive that excess gene copies similar to the copies of our units are also produced in the cell nucleus, but have no opportunity of being incorporated by "infection" into the chromosomal continuum because of a more rigid integration of the genes in the chromosome (needed to meet the requirements of the sexual process) than of the units in the virus particle. Excess pro-

duction of full or partial gene copies has been suggested both on the basis of cytological studies[13] and in connection with theories of gene action.[14]

The transfer mechanism appears to represent a novel, and, in its way, rather efficient means of effecting genetic recombination between virus particles.

Our results thus offer direct evidence for interpreting inactivation of viruses by radiation as due to lethal mutations, and strengthen the concept of a fundamental similarity between viruses and the genetic material of other organisms. They indicate that a virus particle may rather be comparable to a gene complex than to an individual gene. A virus particle can undergo a number of independent mutations,[2, 15] a property that may be shared by certain "gene complexes," defined as cross-over units resolvable into two independently mutable units.[16] The independent mutability of these subgenic units is, however, somewhat doubtful.[16] The virus particle might better be compared to a chromosome, although it behaves more as a unit toward x-rays than the latter. The self-reproducing components seem to possess in the virus particle a degree of solidarity intermediate between that of the components in the supposed gene complex and that of the genes in the chromosome.

There are obvious similarities between the transfer phenomenon here described and other phenomena in which a determinant of heredity is transferred into a new genetic complex, as in the case of type transformation in pneumococci.[17] Phage reproduction may be a particularly favorable material on which to analyze the mechanisms involved.

One interesting application is the possibility of analyzing differences between related but independent wild-type phage strains which can be "crossed" (like the T-even group) in terms of differences in the number of units that can be exchanged (shared loci). Preliminary work in this direction appears promising.

Summary.—Inactivation of large bacteriophages by ultraviolet light is due to lethal mutations at a number of different loci. Each of these loci appears to be independently transferable from one phage particle to others inside the same bacterium. The transfer of loci between irradiated inactive particles results in formation of active phage if the infecting particles, taken as a group, possess at least one copy of each locus in active form. The number of loci can be calculated for each phage, and is at least 30 to 50 for some of them. Phage growth appears to take place by independent reproduction of each of these "unit loci" inside the host cell.

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[7] Formula (1) was derived as follows:

Probability that a given locus receives no lethal mutation $= e^{-r/n}$.
Probability that a given locus receives a lethal mutation $= 1 - e^{-r/n}$.
Probability that a given locus receives a lethal mutation in all of k particles $= (1 - e^{-r/n})^k$.
Probability that a given locus does not receive a lethal mutation in all of k particles $= 1 - (1 - e^{-r/n})^k$.
Probability that none on n loci receives a lethal mutation in all of k particles $= y_k = [1 - (1 - e^{-r/n})^k]^n$

[8] It should be noticed that x (average number of phage particles adsorbed per bacterium) is different from k (actual number of phage particles adsorbed by a given bacterium). The proportion of cells with k particles is $(x^k/k!)\, e^{-x}$.

[9] These results differ from earlier observations[5] of interference between inactive and active particles of the same phage. The older experiments, however, were done under conditions not comparable with those discussed here.

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*NUTRITIONAL LIFE HISTORY AS INFLUENCED BY DIETARY ENRICHMENTS. I.**

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Communicated June 20, 1947

Starting with a basal diet of natural foods which is adequate in the sense and degree that rat families are thriving upon it in the 63rd generation, the effects of enriching the diet (*a*) with protein in the form of poultry meat, and (*b*) with added calcium also, are being studied.

These enrichments in protein increase the rate of growth and result in earlier puberty. With females, however the records from puberty to middle age are variable when the dietary is enriched in protein food only. They raise the question whether the more rapid growth and earlier sexual

maturity are advantageous to all individuals, when induced by dietary enrichment essentially in protein alone. That the average differences thus induced are significant is shown by the data of tables 1 and 2.

TABLE 1

AVERAGE DIFFERENCES IN BODY WEIGHTS OF RATS ON BASAL DIET ALONE AND WITH ADDED PROTEIN (10 RATS IN EACH CASE)

AGE, DAYS	ON BASAL DIET (DIET 16) ONLY, GRAMS	ON BASAL + PROTEIN (DIET 16p5) GRAMS	DIFFERENCE ± ITS P. E.* GRAMS	CRITICAL RATIO
		MALES		
28	49.9 ± 1.5*	49.1 ± 1.4	0.8 ± 2.1	0.4
35	58.1 ± 2.1	67.1 ± 2.5	9.0 ± 3.3	2.7
56	98.4 ± 3.8	130.6 ± 2.9	32.2 ± 4.8	6.7
84	153.9 ± 4.9	202.2 ± 3.2	48.3 ± 5.9	8.2
		FEMALES		
28	46.4 ± 1.5	46.8 ± 1.4	0.4 ± 2.1	0.2
35	54.0 ± 1.9	61.2 ± 2.1	7.2 ± 2.8	2.6
56	86.1 ± 2.4	108.2 ± 1.9	22.1 ± 3.1	7.1
84	119.5 ± 2.4	158.5 ± 2.9	39.0 ± 3.8	10.3

* This ± precision measure is the classical Probable Error.

With both sexes it is clear that the data of table 1 show equivalence at the starting point of 28 days of age (conventional "end of infancy" in the rat) and increasing divergence, due to the greater growth on the diet of higher protein content, up to the age of 84 days. Beyond that age the difference diminishes as the animals on both diets are approaching the normal average adult size.

Table 2 shows the mean differences in several aspects of the early breeding records of females on the two diets. At every point the extra protein has here accelerated the result.

TABLE 2

AVERAGE RECORDS OF 10 FEMALES EACH ON BASAL DIET ALONE AND WITH ADDED PROTEIN. ALL THESE RECORDS ARE TO THE AGE OF 210 DAYS ONLY.

	ON DIET 16 (BASAL ONLY)	ON DIET 16p5 (ADDED PROTEIN)
Age at birth of first young, days	132	98
Total number of litters borne	19	28
Total number of young borne	123	174
Young reared to the age of 28 days	70	146
Total weight of young at 28 days of age, grams	2481	5786
Average weight of young at 28 days of age, grams	35.4	39.6

The foregoing are representative of the majority of our experiments, with matched groups of litter-mate controlled animals, showing accentuation of growth and of early reproduction as results of the enrichment of the basal ration with protein in the form of poultry meat.

In another comparison of the same kind, however, three out of thirteen females have broken down in or after early reproduction and with the development of a nervous condition suggestive of relative calcium deficiency. Autopsies of two of them showed no major infections while chemical analysis of the third showed only 0.68 per cent of body calcium upon death at 169 days as compared with averages of 0.95 to 1.15 per cent found in animals of the same age which had subsisted on the basal diet without supplement. The occurrence of such a low calcium condition is confirmed in other analyses of experimental animals from comparable diets.

In a further series of experiments, the same protein supplement is being fed with basal diets of different calcium contents. The rat families of more liberal calcium intake have been (and are) entirely free from any sign of a period of imbalance, and show higher vitality than their litter-mates and cousins on lower-calcium dietaries. This may or may not appear in the numerical data of growth and development; but is visible in their alertness and general appearance, and palpable in their superior muscle tone and firmness of subcutaneous tissue.

Thus a protein enrichment which, when added alone to a rather low calcium diet, may merely stimulate growth and early puberty at some cost of good balance and stability of the nervous system, appears to be freed from this hazard of imbalance when the basal dietary is of higher calcium content.

Experiments with diets of both the above-described types are being continued, and we hope to report later upon their relations to nutritional well-being in the later segments of the life cycle.

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*FURTHER STUDIES OF THE INFLUENCE OF NUTRITION UPON THE CHEMICAL COMPOSITION OF THE BODY**

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Previous papers from this laboratory have described experiments with rats fed a basal diet consisting of natural foods (Diet A: Laboratory No. 16) which diet has shown itself adequate to the support of normal health, growth, and reproduction generation after generation, yet can be improved either by shifting the proportions of foods it contains or by enrichment with certain specific nutrients.[1-5] Independently of other change in this basal diet, addition of calcium[2-4] or of vitamin A increases the body's con-

tent of the respective nutrient and also improves the nutritional life history.

Corresponding enrichments of the same basal diet with riboflavin and protein might, however, be expected to act interdependently (rather than independently), consistently with the view that the body holds riboflavin in combination with protein, and with the general mass-action or concentration principle. This has been found to be the case.

In one series of five comparisons, rats of the same genetic and nutritional background were fed diets alike in other respects and containing the same liberal amount of riboflavin (7 mcg. per gram of air-dry food mixture) at two levels of protein intake. The average results were: (*a*) with 12 per cent of protein in the food, 5.70 mcg. of riboflavin per gram, and 16.95 per cent of protein, in the body; (*b*) with 32 per cent of protein in the food, 6.63 mcg. of riboflavin per gram, and 18.42 per cent of protein, in the body. Thus when the protein content of the food was varied as widely as from 12 to 32 per cent, the increased protein intake raised the body's concentration levels of both protein and riboflavin by about one-tenth. These findings thus confirm and extend those of other investigators[6, 7] whose experimental work, largely simultaneous, entered the field at different angles of approach from ours.

In other experiments to be reported in full elsewhere, we have compared the effects of diets containing, respectively, 1, 3, and 9 mcg. of riboflavin per gram. When the protein content of the diet was 12 per cent, the percentages of body protein found at these three riboflavin intake levels were 16.41, 16.90, and 17.22, and the amounts of riboflavin per gram of body were 6.11, 6.95, and 8.14 mcg., respectively. When the diet contained 20 per cent of protein the effect of the same variation of riboflavin intake upon body composition was less. The respective percentages of body protein were 18.00, 18.20, and 18.60, indicating that at an intake level of 20 per cent of food-protein, the body-protein content was practically upon its plateau level. At this same protein intake level, the corresponding amounts of riboflavin in the body were 6.04, 6.67, and 7.17 mcg. per gram, indicating that the plateau level of the body's riboflavin content was reached with intake levels of about 3 to 7 mcg. per gram of food according to the experimental conditions.

In contrast to the mutually favorable effects of protein and riboflavin, the acceleration of growth by relatively high protein food may result in retardation of the body's normal developmental gain in calcium content. This was noticeable in our experimental enrichment of a basal diet of about minimal adequate calcium content by the addition of meat, and has also been found in parallel enrichments with pure casein so that it appears to be essentially a protein effect. Or it may be regarded as an effect of enhanced growth, which in these cases was induced by the feeding of extra protein.

Further experiments upon the relation of protein-augmented growth to the calcium content of the body at different ages are in progress and we plan to publish additional findings later. We have, however, no desire to "reserve" this field research. Rather, we are publishing this brief account now in the hope that other investigators of high protein feeding may study further, and from various angles, the indication that the body's ability to make good use of high protein diets depends largely upon such diets possessing also a liberal calcium content.

* Aided by grants from the Carnegie Institution of Washington and from the John and Mary R. Markle Foundation.

[1] Sherman, H. C., and Campbell, H. L., *J. Biol. Chem.*, **60**, 5–15 (1924); *Proc. Nat. Acad. Sci.*, **14**, 852–855 (1928); *J. Nutrition*, **2**, 415–417 (1930); **14**, 609–620 (1937).

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[4] Lanford, C. S., Campbell, H. L., and Sherman, H. C., *J. Biol. Chem.*, **137**, 627–634 (1941).

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CYTOLOGICAL PHENOMENA AND SEX IN HYPOMYCES SOLANI F. CUCURBITAE

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Hypomyces solani f. *cucurbitae* S. & H. is a hermaphroditic, self-incompatible fungus (Hansen and Snyder, 1943). However, male and female strains of the same organism have also been found. In a recent paper (1946) Hansen and Snyder describe the interesting sex-behavior of this fungus.

Perithecia will be produced only when conidia from either a male or a hermaphroditic strain are placed on perithecial primordia of either a female or a hermaphroditic culture of the opposite compatibility group. The male strain differs from both the female and hermaphrodite by the absence of perithicial primordia and sporodochia, while the female, although resembling the hermaphrodite in cultural characters, cannot act as the fertilizing agent.

When the different crosses are analyzed by means of single ascospore cultures it is found that the progeny of the combination, hermaphrodite × male, consists of 50% males and 50% hermaphrodites. The same 1:1

relationship holds for the ascospore progeny of the cross, female × hermaphrodite, while the combination, hermaphrodite × hermaphrodite, gives hermaphrodites only. However, when a female is crossed with a male, the progeny will show, apart from the parental types, a certain number of hermaphrodites and a certain number of neuters. The latter are strains which are completely unable to react sexually either as female or male. These unexpected types, hermaphrodites and neuters, appear in about the following ratio: 3 males: 3 females: 1 hermaphrodite: 1 neuter. This phenomenon was explained (Hansen and Snyder, 1946) by the assumption that the genes for male and female sex are not alleles but occupy different loci in homologous chromosomes, and that crossing over takes place between them.

In the hope of obtaining a cytological explanation for these phenomena, the nuclear condition obtained in the various crosses was studied. Perithecia which had not yet begun to exude ascospores were fixed in a modified Carnoy's solution (1 part glacial acetic acid, 2 parts absolute alcohol, 3 parts chloroform) for at least 48 hours, then smeared in a drop of acetocarmine containing a liberal amount of iron. A coverslip was then added, the slide heated and the preparation pressed flat. Strains of the fungus differed considerably in their ability to take up the strain.

Fusion of the parental nuclei takes place in the young ascus, and as soon as the latter begins to enlarge the fusion nucleus begins its meiotic division. The prophase is protracted and reaches its close only when the ascus has attained nearly full size. The prophase chromosomes are large compared with their small metaphase size, but since they are entangled and do not stain well it was only in a few cases that their number could be definitely determined at the prophase stage. In these cases, however, something of their morphology could also be made out. Beginning with the first prophase the chromosomes become progressively smaller; in the second metaphase they are already very minute, but since the chromosomes in these later stages stain better they could more readily be counted.

It was found that in the cross female × male the diploid nucleus has three pairs of chromosomes which can easily be distinguished. The two members of the first pair are relatively large and slightly heterobrachial; those of the second are a little more than half as long and those of the third about one third the size of those of the second. The largest chromosome has a satellite region which in some prophase preparations appears to be attached to the nucleolus.

However, when the cross hermaphrodite × hermaphrodite was investigated it was found that four pairs of chromosomes were present. One pair was relatively large, another very small, as in the above cross, but here there were two pairs of medium sized (chromosomes) which were essentially

alike, except that in some preparations the members of one pair appeared to be slightly curved while those of the other were straight.

The cross hermaphrodite × male showed seven diploid chromosomes. In the first anaphase three of these migrated to one pole while the remaining four moved in the opposite direction. The same was seen in preparations of asci derived from fertilization of a female by a hermaphrodite.

Thus a hermaphrodite haploid would have four chromosomes (one large, two medium and one small), while a male or a female would have only three (one large, one medium and one small). But how do the hermaphrodite and the neuter types arise from the cross female × male? In a few preparations it appeared that the six diploid chromosomes separated unequally, 4 going to one pole, only two to the other. This would mean that the two medium sized chromosomes, one of which contains the factor for male sex, the other for female, fail to separate in a certain number of cases (to judge from the genetical data) and both move to one pole. The spores formed from the nucleus containing only two chromosomes will be neuters.

The results obtained here provide an explanation for the derivation of male, female and even neuter clones from a hermaphroditic fungus. They also demonstrate that the haploid chromosome number in this fungus may be two, three or four.

These cytogenetic studies in Hypomyces are being continued by the writer.

* The work reported here was done under the direction of Prof. H. N. Hansen and Prof. W. C. Snyder of the Division of Plant Pathology, University of California, Berkeley, California.

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MULTIPLY VALUED HARMONIC FUNCTIONS. GREEN'S THEOREM

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1. The Multiple-Leaved Domain.—A multiple-leaved space $\mathfrak{M}$ in three dimensions is the analog of a Riemann surface in the plane. Let T be a bounded domain on $\mathfrak{M}$, whose boundary consists of a finite number of closed branch curves $s_{(1)}, \ldots, s_{(r)}$ and a bounded exterior frontier T^*, in

such a way that the situation is equivalent topologically to an m-leaved sphere in which the $s_{(i)}$ correspond to branch circles within the sphere, no two of which loop or have points in common. We assume that the $s_{(i)}$ are of zero capacity, considered as closed point sets in space. For our purposes there is no essential loss of generality if we restrict ourselves to a single branch curve s, connecting cyclically all m leaves. We are specially interested in the rôle of the branch curve, and since the boundary T^*, as described by means of the topologically equivalent image space, is not involved by it but lies on m separate leaves of $\mathfrak{M}$, we may take the parts $T_{(1)}^*, \ldots, T_{(m)}^*$ of the boundary to be regular surfaces.

THEOREM. *Let $u(M)$, $v(M)$ be two functions, bounded and harmonic in T and on T^*. Then, with n as the exterior normal to T^* at P,*

$$\int_{T^*}\left(u\frac{dv}{dn} - v\frac{du}{dn}\right)dP = 0, \tag{1}$$

$$\int_{T^*} u\frac{dv}{dn}dP = \int_T (\text{grad } u \cdot \text{grad } v)dM. \tag{2}$$

The essence of the theorem is that there is no contribution to the boundary integrals from the branch curves themselves. The proof of (1) is simpler than that of (2), although, of course, (1) is a consequence of (2).

In a paper which dealt primarily with multiple-valued Green's functions in the case where the branch curves were infinite straight lines, Sommerfeld[1] gave incidentally a proof of (2), assuming, however, that the branch curves were sufficiently smooth and that $u(M)$ was continuous on the branch curves. But this limitation is awkward and disguises the essential nature of the theorem. Examples show that continuity on the branch curves cannot be specified in advance.[2] It is essential, however, that the function be bounded and that the branch curves be sets of zero capacity, because if either of these restrictions is eliminated the theorem is no longer valid. The behavior at the branch curves tends to be determined by the geometric character of the domain rather than by the individual function.

The multiple-leaved space $\mathfrak{M}$ lies on a univalent base space, and the domain T lies on a univalent domain $\bar{T}$ composed of all points whose coördinates $(\bar{x}, \bar{y}, \bar{z})$ are the same as the coördinates of any point of T. We denote by barred symbols, in this way, the projection on the base space of a given configuration on $\mathfrak{M}$. A point Q of the branch curve s is defined to be a *limit point* of a set E of points on $T + s$, if $\bar{Q}$ is a limit point of $\bar{E}$.[3] With this definition, it is seen that any infinite set on $T + T^* + s$ has a limit point on $T + T^* + s$.

2. *Proof of (1).*—The development of (1) may be indicated briefly. Construct a sequence of approximating domains T_k to T with boundaries T^* (fixed) and t_k (variable), the latter being multivalent tori which isolate

the s. The t_k may be obtained as images of tori in the equivalent topological space, and then smoothed out into regular surfaces by a well-known process. We may now determine a corresponding sequence of functions $u_k(M)$, $v_k(M)$, bounded and harmonic in T_k, and taking on the same values as $u(M)$, $v(M)$, respectively, on T^* and zero values on t_k. These functions are constructed by means of a generalization of the Schwarz alternating process. Since their behavior at a point of t_k or T^* depends merely on these boundaries locally, they are entirely regular at such points, and Green's theorem may be applied to T_k. Hence

$$\int_{T^*}\left(u_k\frac{dv_k}{dn} - v_k\frac{du_k}{dn}\right)dP + \int_{t_k}\left(u_k\frac{dv_k}{dn} - v_k\frac{du_k}{dn}\right)dP = 0.$$

But the last integral vanishes, through the definitions of u_k, v_k. Let then k become infinite. It is seen without difficulty that the u_k, v_k converge to functions $U(M)$, $V(M)$ harmonic in T and on T^*, and bounded, the convergence being uniform in the neighborhood of T^*. Hence

$$\int_{T^*}\left(U\frac{dV}{dn} - V\frac{dU}{dn}\right)dP = 0.$$

But also it is seen that U, V take on the same boundary values as u, v, respectively, on T^*. We shall see that they are identical with u, v in T, and in this way we shall obtain the identity (1).

In fact the function U-u vanishes on T^*, and is bounded and harmonic in T, and if it is not identically zero, either it or its negative is positive somewhere in T. Let $w(M)$ denote this choice of U-u or u-U. Then $w(M)$ has a positive upper bound B in T. We shall see that this is impossible on account of the hypothesis that s is of zero capacity.

3. Kellogg's Theorem on the Upper Bound.[4]—The theorem of O. D. Kellogg, on the capacity of sets on the boundary associated with the upper bound of a harmonic function, may be adapted to multiply valued functions, and, incidentally, applied to subharmonic functions.[5]

KELLOGG'S THEOREM. *Let $w(M)$ be subharmonic (in particular, harmonic) in the bounded domain T on $\mathfrak{M}$ and possess the finite least upper bound B in T. With $\epsilon > 0$, let e be the set of boundary points Q (that is, Q on T^* or on a branch curve) where*

$$\limsup_{M\to Q} w(M) \geq B - \epsilon, \quad M \text{ in } T.$$

Then the base set $\bar{e}$ of e is closed and of positive capacity.

The proof follows closely the method of Kellogg. It will be noted that since the branch curves are of zero capacity the portion of e on the exterior boundary $T_{(i)}{}^*$ for at least one of the leaves $T_{(i)}$ must be of positive capacity. This fact insures the uniqueness of bounded harmonic functions

in general as determined by their boundary values on T^*, without regard to their values on the branch curves, and in particular completes the proof of (1).

4. *Proof of (2).*—We consider first the summability over T of $(\text{grad } u)^2$ and the proof of the identity

$$\int_T (\text{grad } u)^2 dM = \int_{T^*} u \frac{du}{dn} dP. \tag{3}$$

The method used for (1) shows that

$$\begin{aligned}\int_{T^*} u\frac{du}{dn}dP &= \lim_{k\to\infty} \int_{T_k} (\text{grad } u_k)^2 dM \\ &= \int_{T_j} (\text{grad } u)^2 dM + \lim_{k\to\infty} \int_{T-T_j} (\text{grad } u_k)^2 dM \\ &= \lim_{j\to\infty} \int_{T_j} (\text{grad } u)^2 dM + \lim_{j\to\infty} \lim_{k\to\infty} \int_{T-T_j} (\text{grad } u_k)^2 dM,\end{aligned}$$

provided that we define u_k, for convenience, as zero in $T - T_k$. A similar identity holds for $\int_{T^*} u\frac{dv}{dn}dP$.

In particular the summability of $(\text{grad } u)^2$ is established, and since $2|\text{grad } u \cdot \text{grad } v| \leq (\text{grad } u)^2 + (\text{grad } v)^2$ the summability of grad $u \cdot$ grad v is also verified. Moreover if (3) is proved it will follow that

$$\lim_{j\to\infty} \lim_{k\to\infty} \int_{T-T_j} (\text{grad } u_k)^2 dM = 0,$$

so that the corresponding limit involving grad $u_k \cdot$ grad v_k will also vanish, and (2) will be proved.

Since we are dealing with a single branch curve s there is no loss of generality in assuming that all the branches $t_{(i)k}$ of the tori t_k have the same base set $\bar{t}_k$, and (by adjoining univalent domains) that all the branches $T_{(i)}{}^*$ of T^* have the same base set $\bar{T}^*$. The symmetric function,

$$V(\bar{M}) = \sum_{i=1}^{m} u_{(i)}{}^2(M),$$

will be bounded and univalent in $\bar{T}$. Moreover, the Laplacian of V will be given by the formula

$$\nabla^2 V(\bar{M}) = 2\sum_{i=1}^{m} (\text{grad } u_{(i)})^2,$$

$u_{(i)}(M)$ being harmonic, and $\nabla^2 V(\bar{M})$ will be summable and $V(\bar{M})$ subharmonic in T. Also $dV/dn = 2\Sigma_i u_{(i)} du_{(i)}/dn$. The identity (3) therefore is equivalent to the identity

$$\int_{\bar{T}} \nabla^2 V d\bar{M} = \int_{\bar{T}^*} \frac{dV}{dn} dP. \tag{4}$$

It suffices to prove the latter.

We may now drop the bars in our notation, since henceforth we deal with univalent functions. The proof of (4) seems to involve considerable detail, but the main steps may be stated in terms of several lemmas. With $\rho(P) = \nabla^2 V(P)/(4\pi)$ and T' a domain which includes T (the erstwhile $\bar{T}$), but in which the properties of $V(M)$ still hold, we define the function

$$V_1(M) = V(M) + \int_{T'} \frac{\rho(P)}{MP} dP$$

which is harmonic in T' and bounded below, since $\rho(P) \geq 0$.

LEMMA 1. *Let F be a closed set of positive capacity and exterior frontier t, surrounded by a domain T of exterior boundary S, the latter being a closed regular surface. There exist two mass distributions $\mu(e)$ and $\nu(e)$, each of one sign, on t and S, respectively, whose combined potential takes on arbitrary constant values, namely, K at all points of S, and N at all points of t which are regular with respect to T. If $N \neq K$ the distribution $\mu(e)$ is not identically zero, and every point of t belongs to the nucleus of $\mu(e)$.*

The proof depends on considerations of minimum intrinsic energy.

LEMMA 2. *Let F and T be as in lemma 1 with the proviso, however, that F be of zero capacity. Let $V_1(M)$ be a function which is harmonic in T and bounded below, defined on F so as to be lower semi-continuous. Then the set $F + T$ is a domain T_1 and $V_1(M)$ is super-harmonic in T_1.* The proof of this lemma involves Kellogg's theorem, lemma 1 and the consideration of the functions $V_1^{(N)}(M)$, obtained by cutting off the function $V_1(M)$ by the arbitrary upper bound N.

We now apply lemma 2 to our problem, replacing F by s (the erstwhile $\bar{s}$), and T by T'. We make use of Riesz's theorem on superharmonic functions in order to deduce that in $T + s$, which is contained in $T' + s$, we may write $V_1(M) = p(M) + h(M)$, where $h(M)$ is harmonic and bounded and $p(M)$ is a potential of positive mass $\mu(e)$ on s. Hence, in $T + s$,

$$V(M) - h(M) = p(M) - \int_{T'} \frac{\rho(P)}{MP} dP, \tag{5}$$

and the right hand member is bounded above.

With the aid again of Kellogg's theorem, this time as applied to univalent subharmonic functions, we can prove

LEMMA 3. *With T and s as above, and s of zero capacity, let $\mu(e)$ be a positive mass distribution on s, with $\mu(s) = 1$, and $\nu(e)$ a positive mass dis-*

tribution on T, with $\nu(T) = \nu < 1$. *Then the difference of potentials* $\int_s d\mu_p/MP - \int_T d\nu_p/MP$ *is unbounded above.*

In (5) the density $\rho(P)$ is summable. Hence the boundedness of its potential in the neighborhood of s depends merely on the values of ρ in a neighborhood of s. Accordingly, with respect to the boundedness of the right-hand member of (5), if $\mu(e)$ were not identically zero, we could discard temporarily as much of the distribution $\rho(P)$ as we pleased, outside that neighborhood, and assume that $\int_{T'} \rho(P)dP < \int_s d\mu_p$. But then, by lemma 3, the right-hand member of (5) would be unbounded above. Since this is not the case, we must have $\mu(e)$ identically zero. Hence

$$V(M) - h(M) = -\int_T \frac{\rho(P)}{MP} dP$$

for M in $T + s$. The function $\rho(P)$, according to its definition, is regular in the neighborhood of T^*. We may therefore apply Gauss's theorem to the univalent function $V(M) - h(M)$. Finally then

$$\int_{T^*} \frac{dV}{dn} dP = 4\pi \int_T \rho(M)dM = \int_T \nabla^2 V dM,$$

which is the equation (4) to be proved.

[1] Sommerfeld, A., "Uber verzweigte Potentiale im Raum," *Proc. London Math. Soc.* **28**, 395–429 (1897).

[2] Evans, G. C., "A necessary and sufficient condition of Wiener," *Amer. Math. Monthly*, **54**, 151–155 (1947).

[3] If there is more than one branch curve a subdomain 3 may be projected on the base space, in order to provide a definition of limit point.

[4] Kellogg, O. D., *Foundations of Potential Theory*, p. 335.

[5] A function is subharmonic in T if it is subharmonic in every univalent domain contained in T.

ON COMPLEXES OVER A RING AND RESTRICTED COHOMOLOGY GROUPS

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The relations between the fundamental group and the homology structure of a space S, developed recently by several authors,[1] may be formulated in a covering space of S as relations between the group of covering transformations (automorphisms) and "restricted" cohomology groups,

based upon cochains with certain invariance properties. In this note we establish a general theory of such restricted cohomology groups in a complex with automorphisms,[2] which among other geometric applications contains these relations as a special case. The group ring of the automorphism group, which is a very useful tool, may be replaced throughout by an arbitrary ring, and the whole algebraic formalism is developed for arbitrary "complexes over a ring."

1. *R-complex.* Let R be a ring with a unit e. A complex $\mathfrak{C}$ over the ring R, in short an R-complex, is a system of R-modules[3] C_n, $n = -1, 0, 1, 2, \ldots$, which are free for $n \geqq 0$, and where for each $n \geqq 0$ an R-homomorphism ∂ of C_n into C_{n-1} is given such that $\partial\partial = 0$. An element $a_n \epsilon C_n$ is called an n-chain; ∂a_n is its boundary and n is the dimension of a_n. Let Z_n be the kernel of ∂, $n \geqq 0$, and $Z_{-1} = C_{-1}$. ∂C_{n+1} is a subgroup of Z_n, and as usual $H_n = Z_n/\partial C_{n+1}$ is called the nth homology group of $\mathfrak{C}$; Z_n and H_n are in an obvious way R-modules.

For a given Abelian group J (called coefficient group) let C^n be the group of all homomorphisms of C_n into J. $f^n \epsilon C^n$ is called an n-cochain; its coboundary $\delta f^n \epsilon C^{n+1}$ is defined by $\delta f^n(a_{n+1}) = f^n(\partial a_{n+1})$ for all chains a_{n+1}. δ is a homomorphism of C^n into C^{n+1}, with kernel Z^n. As $\partial\partial = 0$ implies $\delta\delta = 0$, δC^{n-1} is a subgroup of Z^n and $H^n = Z^n/\delta C^{n-1}$, $n \geqq 0$, is the nth cohomology group of $\mathfrak{C}$ (with coefficient group J). When necessary, it will be denoted explicitly by $H^n(\mathfrak{C})$ or $H^n(\mathfrak{C}, J)$.

2. *Restricted cohomology groups.* Let ψ be a given group of additive homomorphisms of R into J. Instead of considering all cochains $f^n \epsilon C^n$ we restrict ourselves to ψ-cochains; that means, to cochains f^n such that, *for each chain $a_n \epsilon C_n$, $f^n(ra_n)$ considered as function of $r \epsilon R$ is an element of ψ.* All ψ-cochains of dimension n form a subgroup C^n_ψ of C^n. The coboundary operator δ maps C^n_ψ into C^{n+1}_ψ; for $\delta f^n(ra_{n+1}) = f^n(\partial ra_{n+1}) = f^n(r\partial a_{n+1})$, considered as function of $r \epsilon R$, is an element of ψ for each chain a_{n+1}. Let Z^n_ψ be the kernel of the homomorphism δ of C^n_ψ into C^{n+1}_ψ. $H^n_\psi = Z^n_\psi/\delta C^{n-1}_\psi$, $n \geqq 0$, is the nth ψ-cohomology group of $\mathfrak{C}$.

Examples of homomorphism groups ψ:

(2.1) ψ = group of all additive homomorphisms of R into J. In this case $C^n_\psi = C^n$, and the $H^n_\psi = H^n$ are the ordinary cohomology groups of $\mathfrak{C}$.

(2.2) We suppose that J is an R-module, and take for ψ the group of all R-homomorphisms of R into J; $f \epsilon \psi$ is given by its value $f(e)$, since $f(r) = r \cdot f(e)$ for all $r \epsilon R$. C^n_ψ is the group of all R-homomorphisms of C_n into J; the H^n_ψ are called *equivariant* cohomology groups of $\mathfrak{C}$ (cf. §4).

In the following three examples we assume that R is the *group ring* (with integer coefficients) of a multiplicative group G. A homomorphism f of R into J is given by its values $f(x)$ for all $x \epsilon G$ and may be considered as an arbitrary J-valued function of $x \epsilon G$.

(2.3) ψ = group of all functions f such that $f(x)$ is constant for all $x \epsilon G$.

(2.4) We suppose that G acts as an operator group on J, and take ψ to be the group of all functions f such that $f(x) = x \cdot f(e)$ for all $x \in G$. (2.3) is a special case of (2.4), (2.4) of (2.2).

(2.5) All functions f which are "almost 0" for all $x \in G$—i.e., such that $f(x) = 0$ except for a finite number of x—form a group ψ; it is different from the trivial one in (2.1) only if G is infinite.

For any homomorphism f of R into J and any $s \in R$ we define f_s by $f_s(r) = f(rs)$ for all $r \in R$. A homomorphism group ψ will be called *translation invariant*, if it contains with f all f_s, $s \in R$. In the ψ-cochain groups of an R-complex nothing is changed, when an arbitrary ψ is replaced by its maximal translation invariant subgroup. All our examples are translation invariant.

3. *The cohomology sequence.* The factor group C^n/C^n_ψ will be denoted by C^n_r and called the *residual* cochain group (relative to ψ) of dimension n. δ induces a homomorphism of C^n_r into C^{n+1}_r, with kernel Z^n_r, and the groups $H^n_r = Z^n_r/\delta C^{n-1}_r$, $n \geqq 0$, are the residual cohomology groups of $\mathfrak{C}$ (rel. ψ).

The injection ι (the identity mapping) of C^n_ψ into C^n induces a homomorphism of H^n_ψ into H^n. The projection π of C^n onto its factor group C^n_r defines a homomorphism of H^n into H^n_r. Furthermore, if $f^n \in C^n$ and $\pi f^n \in Z^n_r$, then $\delta f^n \in C^{n+1}_\psi$; and if $g^n \in C^n$ such that g^n and f^n are in the same class mod. C^n_ψ, then $\delta g^n - \delta f^n \in \delta C^n_\psi$; thus δ defines a homomorphism of H^n_r into H^{n+1}_ψ. It is easy to see that the homomorphism sequence

$$(3.1)\quad H^0_\psi \to \ldots \to H^n_\psi \xrightarrow{\iota} H^n \xrightarrow{\pi} H^n_r \xrightarrow{\delta} H^{n+1}_\psi \to \ldots$$

is exact; i.e., that the image group of any homomorphism of the sequence is the kernel of the following one.

The sequence of cohomology groups (3.1) together with the kernels (or image groups) of the homomorphisms will be called in short the *cohomology sequence* of $\mathfrak{C}$ (rel. ψ). A mapping (homomorphism, isomorphism) of a cohomology sequence into another one is a mapping of all groups of the first into the corresponding groups of the second. Two cohomology sequences are isomorphic if all groups of the first are isomorphic to the corresponding groups of the second.

A certain subgroup of H^n_r will play a rôle later. All cochains $f^n \in C^n$ such that for each $z_n \in Z_n$ (not necessarily for each chain a_n) $f^n(rz_n)$ considered as function of $r \in R$ is an element of ψ, form a subgroup $\tilde{C}^n$ of C^n. Obviously $C^n_\psi \subset \tilde{C}^n$, and $\tilde{C}^n/C^n_\psi = \tilde{C}^n_r$ is easily seen to be a subgroup of Z^n_r and to contain $\delta\tilde{C}^{n-1}_r$. $\tilde{C}^n_r/\delta\tilde{C}^{n-1}_r = \tilde{H}^n_r$ is the subgroup of H^n_r in question. If $H_n = 0$, $\tilde{H}^n_r = H^n_r$.

4. *Complex with automorphisms.*[2] Let K be a closure finite complex in the sense of combinatorial topology, which admits a group G of automorphisms without fixed cells; in other words, K is a covering of a complex k, and G the corresponding group of covering transformations. When R denotes

the group ring of G with integer coefficients, the groups C_n, $n \geqq 0$, of all finite integer[4] n-chains of K are free R-modules. We define the boundary of a 0-cell to be $= 1$ and understand by C_{-1} the additive group of integers, turned into an R-module by the operation $x.1 = 1$ for all $x \in G$. Then the sequence of all C_n together with the boundary operator ∂ of K forms an R-complex in the sense of §1; we denote it also by K. Its groups H_n are the ordinary integer homology groups of K, $n \geqq 1$. H_0 is a subgroup of the usual 0-dimensional homology group of K; $H_{-1} = 0$.

A cochain f^n in that R-complex is given by its values for all cells of K and may be considered as a J-valued function of these cells. Let ψ be a group of J-valued functions $f(x)$, $x \in G$ (or a group of additive homomorphisms of R into J, cf. §2). f^n is a ψ-cochain if for each n-cell c_n of K the function $f^n(xc_n)$ of $x \in G$ is an element of ψ. The set of all cells xc^n, $x \in G$, for a certain cell c_n, is called a "transitivity domain" of G. When the group ψ is translation invariant, then in order that f^n is a ψ-cochain it is sufficient that the property "$f^n(xc_n)$ is an element of ψ" holds for *one* (arbitrary) cell c_n of each transitivity domain; we say in short that f^n is on each transitivity domain an element of ψ (or "of the type ψ"). Examples: In the case of the group ψ defined in (2.3) a ψ-cochain f^n is constant on each transitivity domain; f^n may be considered as an ordinary cochain in k (for there is a one-one correspondence between the transitivity domains in K and the cells of k), and $H^n_\psi(K)$ is isomorphic to the ordinary cohomology group $H^n(k)$. When G is an operator group of J and ψ given by (2.4), the ψ-cochains f^n are such that $f^n(xc_n) = x \cdot f^n(c_n)$ for all $x \in G$; the "equivariant" groups[5] H^n_ψ may be interpreted as cohomology groups of k, where the ordinary incidence numbers are replaced by operators of J (or, when k is a geometric complex, as cohomology groups of k with local coefficients in the sense of Steenrod[6], for a suitable choice of these local coefficients). When ψ is defined as in (2.5), a ψ-cochain is almost 0 (i.e., finite) on each transitivity domain and will be called *G-finite;* the resulting G-finite cohomology groups of K will be studied in a subsequent note.

5. *ψ-cohomology in an abstract group.* For an abstract group G we denote[7] by $\bar{K}_G$ the following abstract closure finite complex: its n-dimensional cells are the systems $(x_0, x_1, \ldots, x_n)$ of $n+1$ elements of G, and the boundary is defined by $\partial(x_0, x_1, \ldots, x_n) = \sum_{i=0}^{n}(-1)^i(x_0, \ldots, x_{i-1}, x_{i+1}, \ldots, x_n)$. By $x(x_0, x_1, \ldots, x_n) = (xx_0, xx_1, \ldots, xx_n)$ G becomes an automorphism group of $\bar{K}_G$ without fixed cells. Hence, when a group ψ of J-valued functions of $x \in G$ is given, the ψ-cohomology groups H^n_ψ of $\bar{K}_G$ are defined; they depend upon G, J and ψ in a purely algebraic way and may be described by means of J-valued functions $f^n(x_0, x_1, \ldots, x_n)$ of $n+1$ variables $\in G$ such that $f^n(xx_0, xx_1, \ldots, xx_n)$ considered as function of x is always an element of ψ. We denote them by $\Gamma^n_\psi(G, J)$.

When ψ is defined as in (2.3), the $\Gamma^n_\psi(G, J)$ are identical with the cohomology groups of G studied—by purely algebraic means—by Eilenberg and MacLane[8].

All homology groups H_n and cohomology groups H^n of $\bar{K}_G$ are easily seen to be $= 0$. From §3 it follows for all $n \geqq 0$ that

$$\tilde{H}^n_r(\bar{K}_G) = H^n_r(\bar{K}_G) = H^{n+1}_\psi(\bar{K}_G) = \Gamma^{n+1}_\psi(G, J).$$

6. *Chain transformation and homotopy.* We consider again arbitrary R-complexes (§1). Chain transformation of one R-complex into another—over the same ring R—and chain homotopy of such mappings are defined as for ordinary complexes.[9] We add only the condition that all involved homomorphisms A of chain groups C_n into other chain groups C'_m—which are again R-modules—have to be R-homomorphisms; consequently (for a given group ψ of homomorphisms of R into J, cf. §2) the dual homomorphisms A^* of the corresponding cochain group C'^m into C^n map C'^m_ψ into C^n_ψ and C'^m_r into C^n_r, and induce homomorphisms of the different cohomology groups which occur in the cohomology sequence (3.1). It follows easily: When the chain transformation W of an R-complex $\mathfrak{C}$ into itself is chain homotopic to the identity mapping of $\mathfrak{C}$, then the dual mapping W^* induces the identical isomorphism of the cohomology sequence of $\mathfrak{C}$ and of the groups $\tilde{H}^n_r$ defined in §3. In the following we shall use a more precise result:

LEMMA (6.1). Let W be a chain transformation of $\mathfrak{C}$ into itself. If there exist for a certain n two R-homomorphisms X of C_{n-1} into C_n and Y of C_n into C_{n+1} such that $Wa_n - a_n = X\partial a_n + \partial Y a_n$ for all $a_n \in C_n$, then W^* induces the identical isomorphism of all groups and kernels of dimension n in the cohomology sequence of $\mathfrak{C}$, and furthermore of $\tilde{H}^{n+1}_r$ and of $(H^{n+1}_\psi)_0$ (i.e., the kernel of the injection ι of H^{n+1}_ψ into H^{n+1}).

7. *Acyclic R-complexes.* The R-complex $\mathfrak{C}$ is said to be acyclic in the dimension n, if $H_n = 0$. The main result of this note concerns R-complexes which are acyclic in the lowest dimensions $n < N$, for a given $N \geqq 0$. Let $\mathfrak{C}$ be such a complex; straightforward construction yields the two following lemmas (which have nothing to do with the cohomology groups):

LEMMA (7.1). Two chain transformations V and W of $\mathfrak{C}$ into itself, which are equal on C_{-1}, are chain homotopic in all dimensions $n < N$; i.e., there exist for these n R-homomorphisms Y_n of C_n into C_{n+1} such that $Va_n - Wa_n = Y_{n-1}\partial a_n + \partial Y_n a_n$ for all n-chains a_n, $n < N$.

LEMMA (7.2). Let $\mathfrak{C}'$ be an R-complex over the same ring R as $\mathfrak{C}$, with R-modules C'_n, $= 0$ for $n > N$. Then an R-homomorphism of C'_{-1} into C_{-1} may be extended to a chain transformation of $\mathfrak{C}'$ into $\mathfrak{C}$.

Now let $\mathfrak{C}$ and $\mathfrak{C}'$ be R-complexes over the same ring R, both acyclic in all dimensions $n < N$, and with R-isomorphic modules C_{-1} and C'_{-1}; we replace all modules C_n and C'_n with $n > N$ by 0. The R-isomorphism of C_{-1}

onto C'_{-1} may be extended to a chain transformation T of $\mathfrak{C}$ into $\mathfrak{C}'$, and the inverse R-isomorphism to a chain transformation S of $\mathfrak{C}'$ into $\mathfrak{C}$. ST is a chain transformation of $\mathfrak{C}$ into itself, on C_{-1} the identity, hence by (7.1) chain homotopic to the identity mapping of $\mathfrak{C}$ in all dimensions $n < N$. By (6.1) $(ST)^* = T^*S^*$ induces the identical isomorphism of the cohomology sequence (3.1) of $\mathfrak{C}$ in all dimensions $n < N$, and of $\tilde{H}_r^N$ and of $(H_\psi^N)_0$. S^*T^* does the same for the corresponding group of $\mathfrak{C}'$. Therefore, S^* induces isomorphisms of all involved groups of $\mathfrak{C}$ onto the corresponding ones of $\mathfrak{C}'$.

THEOREM (7.3). *If two R-complexes over the same ring R and with R-isomorphic modules C_{-1} are acyclic in all dimensions $< N$, then their cohomology sequences (rel. ψ) are isomorphic in these dimensions, and furthermore their groups $\tilde{H}_r^N$ and their groups $(H_\psi^N)_0$ are isomorphic. This holds for any given group ψ of homomorphisms of R into the coefficient group J.*

Remarks.—(*a*) In many cases $H_n = 0$ for $n < N$ implies $H^n = 0$ for $n < N$; e.g., in a complex with automorphisms (§4). Then, in the cohomology sequence (3.1) ι and π become 0-mappings for $n < N$, δ an isomorphism of H_r^{n-1} onto H_ψ^n for $n < N$ and of H_r^{N-1} onto $(H_\psi^n)_0$. Our result reduces to the fact that the corresponding group H_ψ^n, $n < N$, $(H_\psi^N)_0$ and $\tilde{H}_r^N$ of the two complexes are isomorphic (or equivalently that their corresponding groups $\tilde{H}_r^n$, $n \leqq N$, are isomorphic).

(*b*) By (7.3), in an R-complex which is acyclic in all dimensions $< N$, the cohomology sequence in these dimensions is determined by the abstract structure of R and C_{-1}; we may call it "the cohomology sequence of R and C_{-1}", for the given ψ. As for any R and C_{-1} there exist R-complexes which are acyclic in all dimensions[10], the cohomology sequence (rel. ψ) of R and C_{-1} is always defined in all dimensions.

8. *Acyclic complexes with automorphisms.* We apply theorem (7.3) to a complex K with automorphisms (§4), with the automorphism group G, acyclic in all dimensions $< N$, and to the complex $\bar{K}_G$ (§5). Then, for any coefficient group J and any group ψ of J-valued functions in G, we obtain for the cohomology groups of K the isomorphisms

$$H_\psi^n \cong \Gamma_\psi^n(G, J),\ n < N,$$
$$(H_\psi^N)_0 \cong \Gamma_\psi^N(G, J),$$
$$\tilde{H}_r^N \cong \Gamma_\psi^{N+1}(G, J).$$

They give *purely algebraic relations between the ψ-cohomology groups of K and the automorphism group G*, which contain all previous results[1] of that type as special cases, in particular those concerning relations between the fundamental group of an aspherical space and its cohomology groups (in the ordinary sense, or with local coefficients, corresponding to the equivariant cohomology groups[5] of the universal covering space).

9. The complete statements and proofs together with the discussion and application of special groups ψ will appear in a forthcoming paper. All results extend in a natural way to the product theory of cochains and cohomology classes. The proof of the topological invariance of the ψ-cohomology groups of a polytope with automorphisms and the definition of ψ-cohomology groups for general spaces may be based upon the singular homology theory.

[1] Hopf, H., *Comm. math. helv.*, **17** (1944), pp. 39–79; Eilenberg, S., and MacLane, S., *Ann. of Math.*, **46** (1945), pp. 480–509; Freudenthal, H., *Ann. of Math.*, **47** (1946), pp. 274–316; Eckmann, B., *Comm. math. helv.*, **18** (1946), pp. 232–282; Eilenberg, S., *Trans. Ann. Math. Soc.*, **61** (1947), pp. 378–417.

[2] Cf. for example, G. de Rham, *Comm. math. helv.*, **12** (1939), pp. 191–211; B. Eckmann, ref. 1, pp. 252–254.

[3] An R-module M is an Abelian group, additively written, which admits R as an operator ring, the unit e of R being the identity operator. M is said to be *free*, if it is generated by elements which are linearly independent (over R). An R-homomorphism h of one R-module M into another is a homomorphism such that $h(ra) = r \cdot h(a)$ for all $r \in R$ and $a \in M$.

[4] The groups C_n could be defined with other coefficients than integers; then R would have to be the group ring over these coefficients.

[5] Investigated in detail by Eilenberg (ref. 1).

[6] Steenrod, N. E., *Ann. of Math.*, **44** (1943), pp. 610–627.

[7] Eckmann, B., ref. 1, pp. 257–264.

[8] *Ann. of Math.*, **48** (1947), pp. 51–78 and 326–341.

[9] Cf., for example, S. Eilenberg, *Ann. of Math.*, **45** (1944), pp. 407–447.

[10] See H. Hopf, ref. 1, p. 42.